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Experimental and Supporting Information

Novel nonadride, heptadride and maleic acid metabolites from the byssochlamic acid producer *Byssochlamys fulva* IMI 40021 – an insight into the biosynthesis of maleidrides

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General experimental LCMS with autopurification system comprising Waters 2767 autosampler, Waters 515 HPLC pump, Waters 2998 Diode Array detector, Waters 2424 ELS detector and Waters Quatro Micro mass spectrometer, equipped with guard pre-column. Analytical column: Phenomenex, Kinetex, 5 μ , C18, 100Å, 250 x 4.60 mm; flow rate 1 ml/min. Preparative column: Phenomenex, Kinetex, 5 μ , C18, 100 Å, 250 x 21.20 mm; flow rate 16 ml/min; gradient programmes (30 min) : (i) **Figure 1**: 40-90%; 0 min. - 5% ACN, 2 min. - 40% ACN, 20 min. - 90% ACN, 22 min. - 95% ACN, 26-30 min. – 5% ACN; (ii) **Figure S1**: 15-60%; 0 min. - 5% ACN, 2 min. - 5% ACN, 20 min. - 60% ACN, 22 min. - 95% ACN, 26-30 min. – 5% ACN. All chromatographic solvents used were of HPLC grade and contained 0.05% of formic acid.

NMR instruments: Varian 400-MR (400MHz), Varian VNMR500 (500MHz), Bruker 500 Cryo (500MHz) or Varian VNMR600 Cryo (600MHz).

HRESIMS data was obtained on: ^aBruker Daltonics micrOTOF II, ^bBruker Daltonics Apex IV FT-ICR instruments.

Optical rotations were recorded using the sodium D line (λ = 589 nm) on a Bellingham and Stanley ADP220 polarimeter.

Producing organism *Byssochlamys fulva* strain Olliver & G. Sm. (IMI 40021) from the CABI culture collection.

Production medium Czapek Dox Broth medium, 5% (v/v) of solution A (sodium nitrate 40g/l, potassium chloride 10g/l, magnesium sulphate heptahydrate 10g/l, ferrous sulphate heptahydrate), 5% (v/v) of solution B (di-potassium hydrogen orthophosphat 20g/l), 0.1% (v/v) of solution C (zinc sulphate heptahydrate), 0.1% (v/v) of solution D (cupric sulphate pentahydrate 0.5g) and 3% (w/v) of D-glucose. The medium was sterilized (120°C, 4h) prior to inoculation.

Fermentation conditions Stock of *B. fulva* was grown Potato Dextrose Agar plates (Sigma-Aldrich). For metabolite production, 500 ml Erlenmeyer flask containing 100 ml of sterile Czapek Dox medium was inoculated with a plug of agar from a stock plate, left untouched on a shelf in a constant temperature room at 25°C for 20-50 days of fermentation before extraction.

Extraction and isolation Liquid culture from multiple flasks was combined and mycelia separated at reduced pressure on a Büchner funnel equipped with a cellulose filter. The filtered culture liquid was then acidified with 35% HCl (0.5 ml per 100 ml of liquid) and extracted twice with ethyl acetate. Organic fractions were combined and concentrated on a rotary evaporator (water bath temperature: 30°C) to yield a crude extract, which was re-dissolved in HPLC-grade MeCN prior to LCMS analysis. All compounds were isolated by preparative LCMS system.

Isolated metabolites

Byssochlamic acid **1** - white solid; $[\alpha]^{19} = +91.7^0$ (2.4 mg·ml⁻¹, CHCl₃), (lit. $[\alpha]^{23} = +101^0$, 2.4 mg·ml⁻¹, CHCl₃)ⁱ; t_R 15.4 min; λ_{max} (LCMS) 210, 250 nm; negative ESIMS(LCMS) m/z 331.6 [M-H]⁻; ¹H and ¹³C NMR data see: Table S1.

10-dihydrobyssochlamic acid **2** - isolated as whitish solid; $[\alpha]^{20} = +45.7^0$ (5.0 mg·ml⁻¹ in acetone); t_R 11.2 min; λ_{max} (LCMS) 211, 260 nm; negative ESIMS (LCMS) m/z 333.6 [M-H], 667.9 [2M-H]⁻; negative HRESIMS^b m/z 333.1344 [M-H]⁻ (C₁₈H₂₁O₆ requires 333.1338); ¹H and ¹³C NMR data see: Table S1.

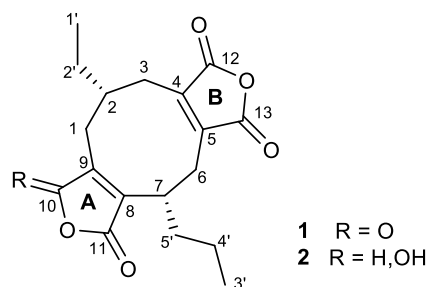
Agnestadride A **17** - whitish solid; t_R 13.2 min; λ_{max} (LCMS) 215, 258 nm; negative ESIMS (LCMS) m/z 165.6, 331.6 [M-H]⁻, 663.9 [2M-H]⁻; negative HRESIMS^a m/z 331.1180 [M-H]⁻ (C₁₈H₁₉O₆ requires 331.1182); ¹H and ¹³C NMR data see: Table S2.

Agnestadride B **18** - bright yellow solid, t_R 18.4 min; λ_{max} (LCMS) 218, 257, 311 nm; negative ESIMS (LCMS) m/z 313.6 [M-H]⁻, 331.7 [M-H+H₂O]; positive HRESIMS^a m/z 337.1053 [M+Na]⁺ (C₁₈H₁₈NaO₅ requires 337.1052); ¹H and ¹³C NMR data see: Table 2.

Anhydride **5** - yellowish oil; t_R 7.6 min; λ_{max} (LCMS) 313 nm; negative ESIMS (LCMS) m/z 165.5 [M-H-CO₂]⁻, 209.5 [M-H]⁻; positive ESIMS (LCMS) m/z 211.5 [M+H]⁺, 193.3 [M+H-H₂O]⁺; ¹H and ¹³C NMR data see: Table S3.

Anhydride **6** - yellowish oil; t_R 14.7 min; λ_{max} (LCMS) 313 nm; negative ESIMS (LCMS) m/z 165.5 [M H]⁻; positive HRESIMS^a m/z 189.0522 [M+Na]⁺ (C₉H₁₀NaO₃ requires 189.0528); ¹H and ¹³C NMR data see: Table S3.

ⁱ J. D. White, K. Jungchul, N. E. Drapela, J. Am. Chem. Soc., 2000, 122, 8665.

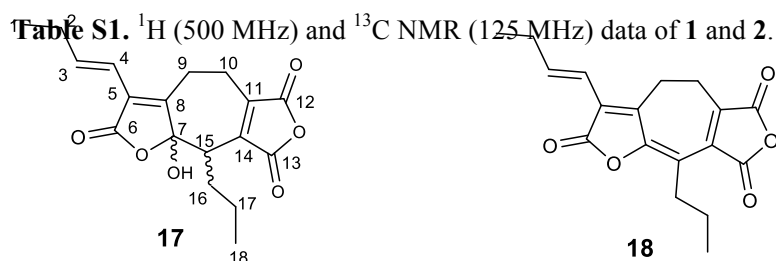


Position	Byssochlamic acid 1			10-dihydrobyssochlamic acid 2		
	δ_H (J in Hz)	δ_C (J in Hz)	HMBC	δ_H (J in Hz)	δ_C (J in Hz)	HMBC
1	2.29 d, 2.72 m	30.2	2,3,2',10,8,9	2.42, 2.32	31.2	2,3,8,9,10,2'
2	1.83-1.98 bs	40.4		1.38	37.2	1', 2'
3	2.63 m, 2.35 m	29.5	1,5	2.89, 2.18	39.3	12,4,5,2,2'
4	-	144.3	-	-	139.1	-
5	-	143.3	-	-	140.9*	-
6	2.85, 2.90 o/m	28.2	4,5,7,13	3.03, 2.60	37.8	13,8,4,5',5,7
7	3.40 m	34.9	6, 8,9,11	2.81 m	33.8	5',8,9, 11
8	-	144.7	-	-	130.1	-
9	-	143.6	-	-	160.3	-
10	-	165.6	-	5.71	95.9	11, 8
11	-	165.4	-	-	171.8	-
12	-	165.4	-	-	169.3	-
13	-	165.0	-	-	168.5	-
1'	1.12 t (7.3)	11.7	2', 2	0.92 t (7.2)	11.3	2,2'
2'	1.64 m, 1.55 m	30.2	1',2,3,1	1.35	30.8	1',3,2
3'	0.95 t (7.3)	13.9	4', 5'	0.87 t (7.3)	13.9	3', 5'
4'	1.43 m, 1.35 m	20.8	3', 7	1.25 m	20.5	3',5',7

5' 1.69 m 36.3 8,3',4',6,7 1.50, 1.46 o/m 37.0 4',6,7,8

Solvents: ^aCDCl₃, ^bdms_o-d₆; o/m – overlapping multiplets, bs – broad singlet; * visible in HMBC

Table S1. ¹H (500 MHz) and ¹³C NMR (125 MHz) data of **1** and **2**.



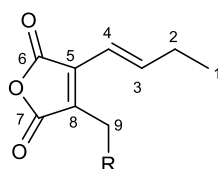
Position	Agnestadride A 17			Agnestadride B 18		
	δ_H (J in Hz) ^a	δ_C (J in Hz) ^b	HMBC ^b	δ_H (J in Hz) ^c	δ_C (J in Hz) ^c	HMBC ^c
1	1.10 (t, 7.5)	13.0	2,3	1.11 (t, 7.4)	13.0	3, 2
2	2.26 (dq)	27.1	1,3,4,5,8	2.30 (dq)	27.5	1,4, 3
3	7.06 (dt, 15.85, 6.6)	143.5	1,2,5	7.12 (dt, 15.86, 6.76)	145.7	1, 2, 5
4	6.08 (dt, 15.85, 1.6)	115.3	2,3,5,6,8	6.18 (dt, 15.7, 1.67)	116.5	5, 2,3,6
5	-	127.0	-	-	126.42	
6	-	168.55	-	-	166.2	
7	-	104.0	-	-	140.0	
8	-	153.4	-	-	142.2	
9	3.01, 2.66 (m)	22.9	5,7,8,10,1 1	1.25, 2.91	29.8 20.9	
10	3.05, 2.52 (m)	20.6	8,9,11,12, 14	2.85		
11	-	143.3	-	-	138.3	
12	-	165.3	-	-	164.6	
13	-	164.8	-	-	163.1	
14	-	144.9	-	-	155.8	
15	3.54 (dd, 10.3, 4.1)	43.8	7,8,11,13, 14,16,17	-	118.7	
16	1.54, 1.22 (m)	31.0	7,14,15,1 7,18	2.86	29.8	

17	1.31, 1.19 (m)	20.8	15,16,18	1.51, 2.86	23.4	16, 18, 15
18	0.88 (t, 7.24)	14.0	17, 16	0.99 (t, 7.4)	14.1	17, 16

Instruments: ^a500MHz, ^b600MHz, ^c500MHz-cryo

Position	Anhydride 5		Anhydride 6	
	δ_H (J in Hz)	δ_C (J in Hz)	δ_H (J in Hz)	δ_C (J in Hz)
1	1.12 (t, 7.4)	12.5	1.12 (t, 7.4)	12.5
2	2.34 (dq, 6.9, 1.6)	27.7	2.32 (dq)	27.6
3	7.32 (dt, 15.8, 6.6)	152.0	7.17 (dt, 15.9, 6.8)	149.1
4	6.24 (dt, 15.8, 1.67)	116.0	6.23 (dt, 16.0, 1.7)	116.2
5	-	139.9	-	137.7

Table S2. ¹H and ¹³C NMR data of **17** and **18** (CDCl₃).



5 R = CO₂ H

6 R = H

6	-	164.0	-	164.9
7	-	165.5	-	166.6
8	-	129.6	-	135.4
9	3.61 (s)	29.6	2.11 (s)	9.3
10	-	172.2	-	-

-

Table S3. ^1H (400 MHz) and ^{13}C NMR (100 MHz) data of **5** and **6** (CDCl_3).

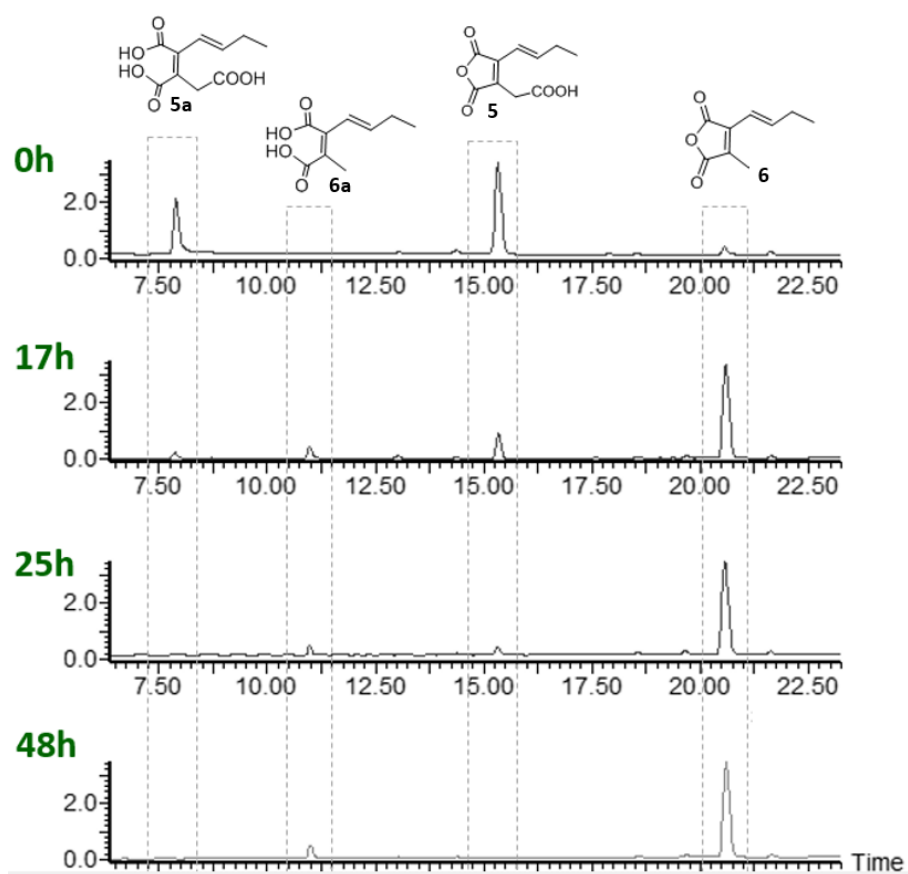


Figure S1. DAD chromatograms showing decarboxylation of anhydride **5** to **6** occurring with time.